Docket No.: 05627-00005-USA
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Design Application of:
A. J. Mixson

Application No.: 10/018103 Art Unit: 1632

Filed: November 5, 2001 Examiner: D. T. Nguyen

For: HISTIDINE COPOLYMERS AND METHODS FOR USING SAME

## Declaration Under 37 C.F.R. § 1.132

Dr. A. James Mixson declares as follows:

1. I am the sole inventor named on the above-referenced patent application.

2. I have read the Office Action dated August 25, 2004.

3. Claims 1-10 and 12-52 have been rejected as lacking enablement in view of a teaching in Midoux (U.S. Pat. No. 6,372,499) that polyhistidine is very poorly soluble in an aqueous medium at neutral pH, and that it is not capable of forming stable complexes with DNA at neutral pH.

4. Exhibit A is a copy of lab notes describing an early experiment that I conducted with a histidine copolymer having a low lysine:histidine ratio of 1:8. A ratio of 1:8 to selected specifically because this low K:H ratio peptide with a high percentage of histidines (89%) would be more likely to precipitate in view of the known poor solubility of polyhistidine. However, I found that this polymer did not precipitate at a pH of approximately 7.0 at two different concentrations. Exhibit A (see notes following the numbers "2" and "3", wherein "ppt." represents the common abbreviation of "precipitation"). In addition, we found that a histidine copolymer:DNA complex formed using this peptide did not precipitate either. Exhibit A (see note following number "5"). Based on these results, this experiment established that a peptide having as high as 89% histidine residues remained soluble.

5. Based on the above results, I would expect that a peptide comprising 90% histidine residues as called for in the current claims would still be soluble.

6. We have since constructed over 80 histidine-lysine (HK) and histidine-arginine (HR) peptides that varied both in their degree of branching complexity and histidine:non-histidine ratios (varying from 1:8 to 1.6:1). All of these polymers are capable of enhancing transfection efficiency although to variable degrees. The molecular weights of these polymers ranged from 1,609 to 22,025 daltons. Exhibit B lists a number of the histidine copolymers having higher percentages of histidine or hydrophobic amino acids located in specific regions. I have not found that any of these polymers are insoluble at physiologic pH (7.2), even at a high concentration of 60 mg/ml. All of the histidine copolymers noted in Exhibit B remained soluble.

7. In addition to the 80 HK and HR peptides, approximately 20 histidine-containing polymers with serine, leucine, and other amino acids have been synthesized without solubility problems.

8. Claims 1-10 and 12-52 have also been rejected as lacking enablement based on a rationale that the specification fails to teach how to associate a transport polymer with pharmaceutical agents other than negatively charged nucleic acid molecules. I disagree with this assertion.

9. The precise method one takes to associating a pharmaceutical agent to a histidine copolymer comprising non-histidine cationic amino acid residues will be obvious to those knowledgeable in the art of organic and medicinal chemistry. There is a large body of literature available on associating small and large pharmaceutical agents to polymers. Consistent with what is known in the art on coupling methods, the specification teaches that covalent bonding of a pharmaceutical agent to a transport polymer is always available as a method for associating these two components regardless of the nature of the non-histidine amino acids present in the transport polymer and regardless of the physical properties of the pharmaceutical agent. See page 13, lines 12-15.

10. In general, the less the negative charge on the pharmaceutical agent, the more likely a covalent bond will be necessary. When the pharmaceutical agent is hydrophobic, then the solubility of the transport polymer:pharmaceutical agent or the type of delivery method (use of a liposome) will dictate the approach to synthesize the final product. If

the pharmaceutical agent is relatively small compared to the cationic polymer, then the solubility issue will not be a problem and this is evident to the skilled artisan.

11. The specification teaches exemplary methods for covalently attaching pharmaceutical agents to transport polymers. See page 17, lines 3-9. As indicated, the specific methodologies mentioned are well known in the art. Other methods of covalently attaching agents to peptides are well known in the art.

12. At pages 6-7 of the Office Action, the Examiner states that a reasonably skilled artisan would not have extrapolated based on the working examples using cationic transport polymer:nucleic acid:liposomal complexes, that other pharmaceutical agent delivery compositions within the scope of the claims would work. Specifically, the Examiner states that "it is not apparent how a skilled artisan, without any undue experimentation, determines as to which order and names of non-histidine amino acids must be specifically arranged in a histidine or polyhistidine containing peptide or polypeptide so as to tailor an intracellular transport of any pharmaceutical agent across the cell membrane. .... Other than the a concentrated emphasis on claimed embodiments covering nucleic acids, the as-filed specification does not provide any guidance as to what is necessary or required for the make [sic] and use of a histidine containing polypeptide that can be used as a generic transport polymer so as to transport an enormous number of pharmaceutical agents other than negatived [sic] charged molecules."

13. I respectfully disagree with the Examiner. While not wishing to be bound by any theory, I note that the method by which the histidine component of the transport polymer facilitates intracellular delivery, is not by enhancing endocytic uptake across the cell membrane, as posited by the Examiner, but by buffering and facilitating release from pre-lysosomal vesicles. One of skill in the art would recognize, in particular with respect to buffering capacity which is unique to histidine among amino acids, that the effect of the histidine copolymer is not due to the nature of the non-histidine amino acids or the physical properties of the pharmaceutical agent used in the working examples.

14. As previously noted, the specification teaches that covalent bonding of pharmaceutical agent to a transport polymer is always available; and that when used as the method of association, does not restrict the nature of the non-histidine amino acids present in the transport polymer. Methods are well known for covalently linking peptides

to other molecules, even in the case of covalently attaching a cationic peptide to a cationic molecule.

15. As would be readily apparent to a skilled artisan based on the specification in view of the knowledge in the art, the selection of non-histidine amino acids is most directly relevant to whether the pharmaceutical delivery composition comprises an intracellular delivery component. In particular, I note that the methods for preparing and using liposomes and micelles to deliver pharmaceutical agents (whether anionic, cationic, neutral, hydrophilic or hydrophobic) were well known in the art as of the filing date of the present application.

16. It would readily be apparent to the skilled artisan based on the physical properties of the transport polymer, pharmaceutical agent, and associated complex thereof, whether an intracellular delivery component would be required. That being said, regardless of the physical properties of the transport polymer and pharmaceutical agent, a skilled artisan could prepare without undue experimentation a pharmaceutical agent delivery composition according to the currently amended claims, which all recite an optional intracellular delivery component (ex. liposomes).

17. Finally, I note that the Examiner has cited Chen *et al.* for the statement that "without liposomes the linear HK [histidine/lysine] polymer would have been discounted as a transfection carrier in both endothelial cells and fibroblast." This statement in Chen *et al.*, of which I am senior author, was made in the context of comparing a linear histidine copolymer with the combination of linear histidine copolymer and liposome as regards *commercial* utility and would have been understood in this manner by one of skill in the art. The statement would not have been interpreted as indicating that a linear or branched histidine copolymer "by themselves" would not have any effect. Indeed, as noted in my earlier declaration, the data indicates that histidine copolymer by itself can enhance transfection efficiency.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATE: 12/21/04

A. James Mixson

Dr. A. James Mixson

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# EXHIBIT A

(attachment to Declaration Submitted Under 37 CFR 1.132)

1:8
lysine hydrochloride

# BECKMAN

9/18/97

Poly-L-lysine

pH of soln - .00008

① Dissolved 2mg in 200 mls of above soln
Test pH of this soln + it was about pH-7

② Took 12 mls + added PBS (7.4) 200uls - no ppt

③ Took 100ul of this soln + 1.7 ug of DNA in 100 mls (min)
no ppt

④ Took 100 mls of this soln + .9 ug of DNA in 100 mls (MIN)

⑤ Took 6 mls of soln 1 in 94 mls of $H_2O$; then
mixed with 1.7 g of DNA in 100 mls,
no ppt.

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# EXHIBIT B

(attachment to Declaration Submitted Under 37 CFR 1.132)

## Selected HK Sequences that have been synthesized and which are soluble

**Non-lysine (or arginine) containing histidine polymers**

1. SHSHSHSHSGSHSHSHSHSHS
2. SHHHHHSHHHHSHHHHS
3. SHGHSHGHSGHSHGHSHG
4. NHNHNHNHNHNGNHNHNHNHN

**Histidine containing polymers with hydrophobic amino acids or (residues)**

5. LLILLILLGHHHSHHHSHHKKK-the most hydrophobic polymer in this group with one end hydrophobic, the other end is hydrophilic; this peptide was synthesized to insert the hydrophobic end of this polymer into the hydrophobic bilayer membrane of liposomes.
6. LLKHKHKHKHKGKHKHKHKHK
7. [KLKHHKHHKHHKHHKHHKHK]$^{4}$KKK (branched polymers; 4 branches off of 3 lysine core)
8 [KLKLHKLHKHHKHHKHHKHK]$^{4}$KKK (branched polymers)
9 [KLKLHKLHKHHKHHKHHKLK]$^{4}$KKK (branched polymers)
10. [KLKLHKLHKHHKHHKLLHKLK]$^{4}$KKK (branched polymers)
11. [KHKHHKHHKHHKHHKHHKHK]$^{4}$KKK* (fluorescein)

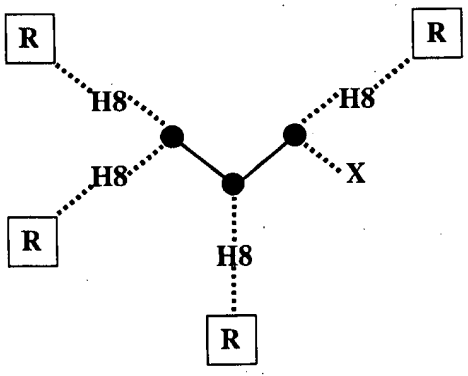
**Branched HK polymers with high histidine percentage**

12. [KHKHHKHHKHHKHHKHHKHK]$^{4}$KKK—*HHHHNHHHHN* (Note that there is a histidine rich domain (italicized) off of the 3 lysine core in this and the next 2 examples)
13. [KHHHKHHHNHHHNHHHK]$^{4}$KKK--*HHHHNHHHHN*
14. [KHHHKHHHNHHHNHHHK]$^{4}$KKK--*HHHHNHHHHN*
15. [KHHHKHHHNHHHNHHHK]$^{4}$KKK

**Highly Branched HK Polymers ($H^3K8b$ and $H^3K(G)8b$) and with histidine-rich domain (H8) and with 8 terminal branches (Polymers 16 and 17).**

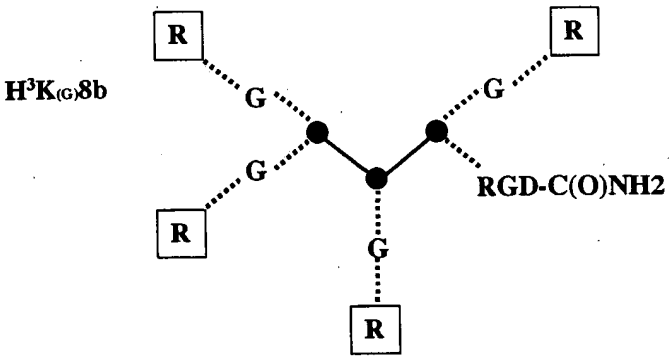
| Polymer | Structure of Branched Polymers | Sequence of Polymer |
|---|---|---|

$H^3K8b$




**H8=H4NH4**
**R=[HHHKHHHKHHHKHHHK]$^{2}$K**
**X=RGD-C(O)NH2**

Percent of Amino Acids for above polymer: K-18.7%; H-77.1; N-2.5; R, G, and D-0.6; Molecular weight is 22,025 daltons. Several similar peptides have been made. .




Percent of Amino Acids for above polymer K-28.2%; H-65.0, G-4.5; R, D-0.9. Molecular weight of this polymer is 14,305 daltons.